50X1-HUM

MAR 1952

CLASSIFICATION C-O-N-F-I-D-E-N-T-I-A-L

CENTRAL INTELLIGENCE AGENCY
INFORMATION FROM
FOREIGN DOCUMENTS OR RADIO BROADCASTS

REPORT

CD NO.

COUNTRY	Czechoslovakia	DATE OF INFORMATION	1953
SUBJECT	Scientific - Biology, microbiology in petroleum prospecting and recovery		
HOW PUBLISHED	Bimonthly periodical	DATE DIST.	18 Apr 1955
WHERE PUBLISHED	Prague	NO. OF PAGES	8
DATE PUBLISHED	Aug 1953		
LANGUAGE	Russian	SUPPLEMENT TO REPORT	

50X1-HUM

THIS IS UNEVALUATED INFORMATION

SOURCE Chekhoslovatskaya Biologiya, Vol 2, No 4, pp 226-231

MICROBIOLOGY IN THE PETROLEUM INDUSTRY

M. Dostalek

[Comment: The article summarized below reviews recent work on the application of bacteriological techniques in the petroleum industry. Although the review compiled by the Czech author is rather objective, it tends to emphasize USSR work on the subject. Much of the information on work done outside of the USSR which is contained in the original article has been omitted in this summary because this information is readily available from other sources.

Dostalek states that microbiological prospecting for petroleum and microbiological surveying of petroleum and gas fields is being applied extensively in the USSR. On the other hand, he does not make any references to USSR applications of the microbiological method for the secondary recovery of petroleum and describes the possibilities of this method solely on the basis of work done in the US by C. E. ZoBell and members of ZoBell's group.

Dostalek gives a high evaluation to the potentialities of the microbiological method and states that extensive and elaborate experimental work is being carried out in order to check the hypothesis of the formation of petroleum from organic debris with the participation of microorganisms. Unless the Czech author's information on US research is out of date, the experimental work to which reference is made is being carried out in the USSR rather than the US, because the theory postulating that formation of petroleum takes place by bacterial degradation seems to have fallen into disfavor in the US. Thus, R. W. Stone and C. E. ZoBell stated in 1952 that there is very little experimental evidence proving that petroleum hydrocarbons constitute the ultimate bacterial end

50X1-HUM

CLASSIFICATION C-O-N-F-I-D-E-N-T-I-A-L

STATE		NAVY		NSRB		DISTRIBUTION	
ARMY		AIR		FBI			

product derived from marine organic matter. At present, research on the role of bacteria in oil formation, which was formerly sponsored by the American Petroleum Institute, has been discontinued (R. E. Kirk, D. F. Othmer, Encyclopedia of Chemical Technology, New York, 1953, Vol 10, p 102). On the other hand, the adherence of USSR petroleum scientists to the "organic" theory of petroleum formation (cf. I. M. Gubkin's views on the subject) and the fact that the microbiological theory of the formation of petroleum is not only regarded as valid in the USSR, but definitely stressed there. -- cf. G. Ye. Ryabukhin, Neft' (Petroleum), Znaniye, Moscow, 1954, 24 pp -- make it very likely that research along the lines indicated in the Czech article is being conducted in that country, possibly under the auspices of the Moscow Petroleum Institute imeni I. M. Gubkin.

Numbers in parentheses refer to author's bibliography.]

Biology is the science which has been applied at a later date than any other to the solution of theoretical and practical problems that have a bearing on the production of crude petroleum. During the past 20 years, petroleum microbiology has developed as an independent discipline which is being applied to an increasing extent in the petroleum industry. The biochemical aspects of petroleum microbiology are determined primarily by the chemical composition of petroleum and natural gases. The microbiology of petroleum is substantially a microbiology of hydrocarbons. The basic biochemical processes which take place in this connection as a result of the action of microorganisms are decomposition of hydrocarbons, formation of hydrocarbons from other substances that contain carbon, and transformation of some types of hydrocarbons into hydrocarbons of other types.

Decomposition of Hydrocarbons

Under appropriate conditions, practically any hydrocarbon can be oxidized by microorganisms and used by the latter as a source of carbon and energy. Not all hydrocarbons are decomposed with equal facility: polyatomic hydrocarbons are decomposed more readily than simple hydrocarbons, paraffinic hydrocarbons more easily than representatives of any other classes, branched and isocompounds more readily than normal paraffins, etc. Although the decomposition of derivatives of hydrocarbons by microorganisms has not been investigated systematically, it has been established that compounds which contain chlorine are not decomposed and that various derivatives of aromatic hydrocarbons, which exert a bacteriostatic or bactericidal action at high concentrations, are not affected at all.

As far as paraffins are concerned, liquid hydrocarbons of this class are decomposed more easily than gaseous hydrocarbons. The adaptive capacity to decompose pentane and higher hydrocarbons of this class has been observed on many common microorganisms. More than 90 species belonging to approximately 20 genera exhibit this type of activity (Schwartz, Mueller -- 1945). The most important genera in question are Pseudomonas, Serracia, Bacterium, Micrococcus, Mycobacterium, Bacillus, Desulfovibrio, Methanomas, Penicillium, Aspergillus, and Torula.

J. Tausz et al (1919) have described interesting organisms of this group and have called them Bacterium aliphaticum and Bacterium aliphaticum liquefaciens. Both of these species decompose liquid paraffinic hydrocarbons. On the other hand, naphthenic hydrocarbons are decomposed only by Bacterium aliphaticum liquefaciens. Tausz et al proposed that these microorganisms be used for the analysis of mixtures or for the separation of paraffinic hydrocarbons from naphthenes.

Gaseous hydrocarbons in the range from methane to propane are decomposed by only a limited number of species of microorganisms and the decomposition of the hydrocarbons by these microorganisms proceeds slowly. Ye. N. Bokova (1) subdivided the microorganisms which oxidize hydrocarbons of this type into three groups: methane-oxidizing bacteria, ethane-oxidizing bacteria, and propane-oxidizing bacteria. The groups are named after the simplest hydrocarbon which is decomposed by members of the group.

The greatest attention has been paid to the investigation of bacteria oxidizing methane.

Solid hydrocarbons are assimilated principally by a number of fungi and also by actinomycetes. Some bacteria decompose paraffin wax. The polymers of olefins and of other hydrocarbons which constitute natural and synthetic rubber are decomposed by fungi, actinomycetes, and bacteria.

Among bacteria that decompose hydrocarbons those which reduce sulfates under anaerobic conditions comprise an important group. The chemistry of the action of these bacteria on hydrocarbons has not yet been clarified completely. In the opinion of some authors, the bacteria in question affect principally paraffinic hydrocarbons beginning with decane and higher than decane. A certain proportion of the hydrocarbon resorbed by the bacteria is oxidized completely, while another fraction is used by them as a source of carbon. In this process lower hydrocarbons are formed, which may be of the naphthene or polymethylene type (J. Appert, 1952, G. D. Novelli; C. E. ZoBell, 1944; W. O. Tauson, 1925).

The end products of the aerobic assimilation of hydrocarbons are, in addition to the organic mass of the bacteria which has formed, primarily carbon dioxide and water. Among the intermediate products of the decomposition of hydrocarbons one finds the most diverse acids, alcohols, oxyphenols, and compounds derived from ketones (C. E. ZoBell, 1946, 1950).

Microorganisms that decompose hydrocarbons are encountered in large quantities in the soil, particularly in the vicinity of petroleum deposits, petroleum pipelines, etc. G. A. Mogilevskiy states that 150,000 bacteria which decompose hydrocarbons occur in one gram of soil (2), while Soehngen (1906) found up to 200,000 of such bacteria in one gram of soil and up to 3,000 of bacteria capable of assimilating paraffin [paraffin wax?] in one milliliter of effluent water.

The Formation of Hydrocarbons

The hydrocarbons which are formed by microorganisms either become a component of their protoplasm or form end products of the microorganisms' metabolism. Methane is formed as a result of the anerobic decomposition of cellulose and of other organic substances. This decomposition is brought about by a number of bacteria, particularly those which form a part of the microflora of the soil. In addition to mesophilic bacteria, a number of thermophilic bacteria are active in this respect. Recently, spectroscopic investigations have demonstrated with certainty that ethane and propane are formed in cultures which are kept at a hydrostatic pressure of 200-500 atmospheres (J. Appert, 1952).

More complex gaseous and liquid hydrocarbons are formed in considerable quantities under the effect of desulfurizing bacteria. It has been shown that paraffinic hydrocarbons of the C_{20} - C_{25} range are formed when certain strains of Desulfovibrio are cultivated under pressure in the presence of caproic acid (C. E. ZoBell, 1934, 1945). The formation of aromatic hydrocarbons as a result of the action of microorganisms has also been proven.

50X1-HUM

C-O-N-F-I-D-E-N-T-I-A-L

As far as investigations in the field of petroleum are concerned, microbiological methods can be applied in the following subdivisions:

1. Microbiological prospecting, correlation of findings, and core sampling

2. The action of microorganisms on hydrocarbons

3. Solution of the problem of petroleum formation

4. Liberation of petroleum from deposits.

Microbiological Prospecting and Surveying

Recently microbiologists have begun to participate in petroleum prospecting. The methods of microbiological prospecting make use of the fact that microorganisms of the soil which require hydrocarbons react in a very sensitive manner to the presence of gaseous hydrocarbons that seep through from petroleum deposits. Although gaseous hydrocarbons often penetrate to the soil's surface in very small concentrations only, the microorganisms close to the surface are very sensitive to them. Ye. N. Bokova states that a content of 0.001% of gaseous hydrocarbons in the air is sufficient to stimulate the propagation of methane-oxidizing or propane-oxidizing bacteria.(1)

Hydrocarbon microorganisms are practically ubiquitous in the soil. However, the most favorable conditions for the propagation of these microorganisms are created by the seepage of gaseous hydrocarbons from [petroleum and natural gas] deposits. Whenever seepage of this kind occurs, accumulation of the microorganisms takes place.

When the number of bacteria which require hydrocarbons is determined in the soil at the right depth, the places where the largest number of bacteria have been found may with a great degree of certainty be assumed to lie above petroleum deposits. G. A. Mogilevskiy et al (2,3), V. A. Sokolov (4), and M. I. Subbota (5,6) developed a method of microbiological prospecting which is founded on this principle. With the aid of this method, many petroleum fields have been discovered in the USSR which show great promise. In many cases petroleum and natural gas deposits have been located by using this method.

To eliminate unfavorable factors which interfere with the application of the microbiological method of prospecting, more perfect procedures have been developed by means of which the presence of gaseous hydrocarbons in the soil is determined with the aid of pure cultures of hydrocarbon bacteria. A culture with a known titer in a suitably prepared inorganic nutrient medium is introduced into shallow wells which are covered on top to exclude air. After a definite period of incubation has passed, the increase in the number of microorganisms in the culture is determined. By using this method, one may determine the relative quantity of gas which seeps through to the spot under investigation and also the composition of this gas, if cultures that are sensitive to specific kinds of hydrocarbons have been used. Information pertaining to the method in question has been published by G. A. Mogilevskiy.(2) A patent pertaining to the subject was taken out by R. T. Sanderson (cf. US Patent No 2294425, issued in 1943).

Characteristic changes occur in the composition of the microflora of waters that have been either in direct contact with petroleum deposits or in contact with gases emanating from such deposits. These phenomena can also be used in prospecting. A method of prospecting by subjecting water to

C-O-N-F-I-D-E-N-T-I-A-L

bacteriological examination has been developed in the USSR. This method is based on regularities governing the accumulation of bacteria which oxidize methane, oxidize liquid hydrocarbons, and reduce sulfates. According to L. D. Shturm, an important factor to be considered in this type of investigation is the morphological composition of the microflora (7). This composition is determined by filtering samples of the water through filters of the film type. (8,3)

Microbiological investigation of samples of stratum waters obtained in the course of drilling is carried out in the same manner. Microbiological correlation of waters occuring in different localities is an essential part of this work. Correlation from the microbiological standpoint is of the greatest importance both for drilling and production. Biological core sampling has also been found useful in recent times. Substantially, this core sampling also amounts to microbiological examination of water and correlation of data obtained in this manner. Of particular importance in the correlation of data are observations pertaining to the accumulation of desulfurizing bacteria.

The significance of microbiology for petroleum prospecting and for the correlation of data pertaining to petroleum prospecting is not limited to the individual methods of investigation mentioned above. The microbiological processes which take place are often so intensive that they affect the results of purely chemical methods of investigation. Thus, methods of gas prospecting that are based on the detection of gaseous hydrocarbons in the soil yield unreliable results in places where bacteria are very active, because the hydrocarbon bacteria are effective as a factor which resorbs and modifies hydrocarbons that seep into the soil. Other chemical methods of prospecting, particularly those which are based on the investigation of organic substances of the soil, are also closely connected with the activity of hydrocarbon bacteria and a knowledge of this activity.(5,6)

Effects of Microorganisms on Petroleum and Petroleum Products

Decomposition of petroleum and of petroleum products by microorganisms may result in considerable damage. Bacteria affect crude petroleum and practically all petroleum products, particularly when water is present (9). Of particular importance is the deterioration of special grades of motor fuel under the action of bacteria. As a result of anaerobic decomposition, precipitation of lead sulfide may take place in gasoline containing tetraethyl lead with consequent reduction of the octane number. Gaseous hydrocarbons which may form as a result of deterioration caused by bacteria increase the danger of spontaneous ignition of liquid fuel that is kept in storage. (J. Appert, 1952; C. E. ZoBell, 1946, 1950).

Hydrocarbon microorganisms cause decomposition of oil in cooling emulsions used in the metallurgical industry, so that other bacteria multiply in the emulsions as a result. Some of these bacteria are pathogenic and induce skin diseases affecting the hands of workers. Other microorganisms of the same class as those which produce the decomposition of oil may cause deterioration of paraffin wax used for sealing bottles which contain medicines and chemicals, with the result that the contents of the bottles also deteriorate. Rubber, both natural and synthetic, likewise deteriorates as a result of the action of microorganisms.

Hydrocarbon bacteria may also exert a beneficial effect. Thus, A. Z. Yurovskiy states that methane-oxidizing bacteria are capable of decomposing up to 96% of the methane contained in the air of coal mines (10). Hydrocarbon bacteria may also be used to advantage to treating industrial wastes that contain hydrocarbons.

Formation of Petroleum

Of the many theories explaining the formation of petroleum, practically only one is being recognized at present, namely the so-called organic theory which assumes that petroleum was formed from organic matter of animal and plant origin. Although it is certain that microorganisms participated in the process of formation of petroleum, their role in this process has not yet been sufficiently clarified.

Petroleum-bearing strata invariably contain live microorganisms, no matter how deep these strata are located (11). The bacteria isolated from petroleum deposits are mostly anaerobes. However, aerobes also occur there and among them representatives of the genus Desulfovibrio are prominent. The strains of microorganisms obtained from great depths are adapted to high pressures, scarcity of oxygen, high temperatures, and other conditions typical for their natural environment. Notwithstanding this, they can be relatively easily accustomed to laboratory conditions of culturing.

Investigation of recent marine sediments has shown that microorganisms which are in every respect close to those found in petroleum deposits take part in the decomposition and transformations of organic matter at the sea bottom. Few aerobes are encountered in marine sediments. Anaerobes predominate there, and among them representatives of the genus Desulfovibric are again prominent. In the process of anaerobic decomposition of organic matter, this matter is enriched in carbon and hydrogen.

As a result of processes taking place at the sea bottom, petroleum dispersed in small quantities over large areas was formed. Formation of the petroleum deposits as we know them today took place by migration of the petroleum that was originally formed. Microorganisms presumably took part in the process of migration (12), as has been suggested by J. Appert and C. E. ZoBell.

A number of experiments that have been carried out with microorganisms isolated from petroleum deposits or from marine silt confirms the correctness of the assumption that microorganisms participate in the processes described above. For instance, it has been established in these experiments that fatty acids may be transformed into liquid hydrocarbons under the action of bacteria. Gaseous hydrocarbons, particularly ethane and propane, may form together with methane under the action of bacteria when the hydrostatic pressure is high. The role of microorganisms in the migration of petroleum has also been confirmed experimentally. A method has even been proposed whereby the effect produced by microorganisms in this respect may be utilized in the secondary exploitation of petroleum occurences. Long-range experiments are being conducted on a large scale with the aim of reproducing completely the process of formation of petroleum from organic debris with the participation of microorganisms.

Liberation of Petroleum from Deposits

It appears to be established that microorganisms are one of the factors which influence the migration of petroleum. The method proposed by ZoBell for a more complete liberation of petroleum from deposits with the aid of a microbiological procedure duplicates to a certain extent processes which occur in nature. In this method cultures of appropriate microorganisms that are sensitive to petroleum hydrocarbons are introduced into petroleum deposits the primary exploitation of which has been completed. These deposits still contain a considerable amount of petroleum adsorbed on matter which consists principally of carbonates. In such deposits the microorganisms propagate intensively and affect both the petroleum and the surrounding rock formations, contributing to the liberation of the petroleum in the following ways:

50X1-HUM

1. As a result of decomposition and evolution of carbon dioxide and methane, the viscosity of the petroleum is lowered.

2. The carbonates are destroyed under the effects of the acids which are formed.

3. The gases that are formed in processes (a) and (b) increase the pressure, in this manner bringing about displacement of the petroleum.

4. Fatty acids and other decomposition products facilitate saponification [sic; presumably should be emulsification] of the oils in water.

The microorganisms which are suitable for this procedure belong to the class of bacteria that reduce sulfates, decompose hydrocarbons, and are resistant to high concentration of salts.

The present review does not cover all possibilities of applying microorganisms in investigations pertaining to petroleum. The microbiology of petroleum is forging ahead and receives the greatest amount of attention everywhere. Moreover, the significance of data on hydrocarbon microorganisms is not restricted to applications in the field of petroleum. These data also have a bearing on many other fields, such as industry, public health, biological purification of water, and investigations of coal deposits.

BIBLIOGRAPHY

1. Ye. N. Bokova, V. A. Kuznetsova, S. I. Kuznetsov, "Oxidation of Gaseous Hydrocarbons as the Basis of Microbiological Prospecting for Petroleum," Doklady Akademii Nauk SSSR, Vol 36, 1947, p 755

2. G. A Mogilevskiy, "The Bacteriological Method of Prospecting for Petroleum," Razvedka Nedr, No 12, 1940, p 32

3. G. A Mogilevskiy, "Ten Years of Geomicrobiological Prospecting" Razvedka Nedr, No 5, 1947, p 48

4. V. A. Sokolov, Pryamyye Geokhimicheskiye Metody Poiskov Nefti (Direct Geochemical Methods of Prospecting for Petroleum), Moscow, 1947

5. M. I. Subbota, "The Problems of Field Methods to Be Used in Prospecting for Petroleum by the Method of Bacteriological Surveying," Razvedka Nedr, No 3, 1947, p 20

6. M. I. Subbota, "Comprehensive Investigation of Seasonal Variations of Data Obtained in Surveying for Gas," Neftyanoye Khozyaystvo, 1947

7. L. D. Shturm, "Microbiological Investigation of Petroleum-Bearing Stratum Waters," Mikrobiologiya, Vol 19, 1950, p 43

8. V. A. Ekzertsev, "Microscopic Investigation of the Bacterial Flora in the Petroleum-Bearing Facies Strata of Second Baku," Mikrobiologiya, Vol 20, 1951, p 324

9. A. A. Voroshilova, Ye. D. Diyanova, "Petroleum-Oxidizing Bacteria as Indices of the Intensity of Oxidation of Petroleum Under Natural Conditions," Mikrobiologiya, Vol 21, 1952, p 408

10. A. Z. Yurovskiy, G. R. Kaplikash, B. V. Mangubi, Ugol', No 7, 1939, p 48

11. B. L. Isachenko, "On the Microorganisms of the Lower Limits of the Biosphere," Journal of Bacteriology, Vol 40, 1940, p 379

12. A. Luchterowa, "Geomicrobiology in the Petroleum Industry," Nafta, Vol 8, No 8, 1952, p 234

13. T. L. Ginzburg-Karagicheva, "Microbiological Investigation of the Sulfur-Salt Waters of Apsheron," Azerbeydzhanskoye Neftyanoye Khozyaystvo, No 6-7, 1926

14. N. B. Nechayeva, "Two Species of Mycobacteria that Oxidize Methane," Mikrobiologiya, Vol 18, No 4, 1949, pp 310-17

15. G. P. Slavina, "Peroxidase in Methane-Oxidizing Bacteria," Doklady Akademii Nauk SSSR, Vol 56, 1947, p 205

16. G. P. Slavina, "Application of the Fluorescence Method in the Investigation of Some Species of Bacteria That Oxidize Hydrocarbons," Mikrobiologiya, Vol 17, 1948, p 76

- E N D -